

Ocular arylalkylamine N-acetyltransferase-1 (AANAT1) gene expression with gonadal development in grass puffer

Marine Science Institute, Jeju National University

Byeong-Hoon Kim

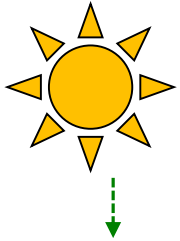
Introduction

❖ Annual spawning season in fish

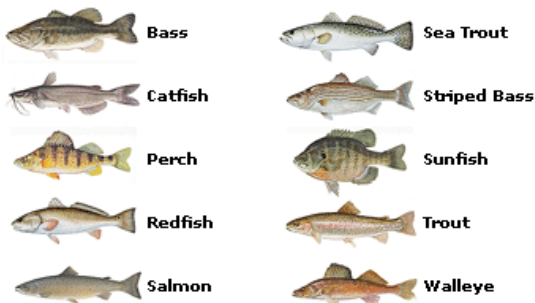
: The every fish species has optimal photoperiod and temperature timing for reproductive activity.

Environmental factors

Photoperiod



Water temperature



Reproductive seasons in Jeju

Spring breeder



Olive flounder

Summer breeder



Damselfish

Autumn breeder



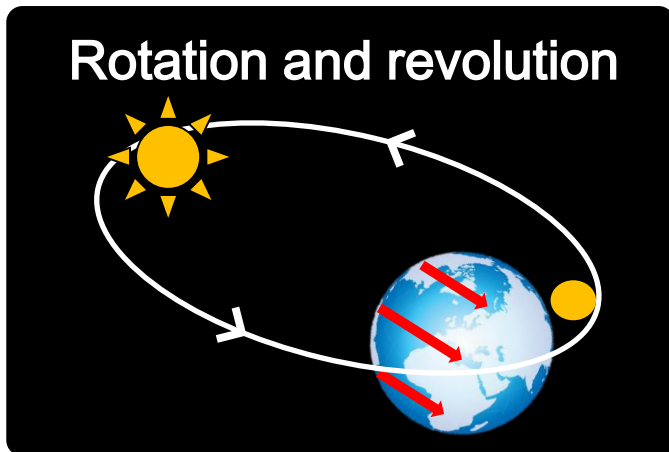
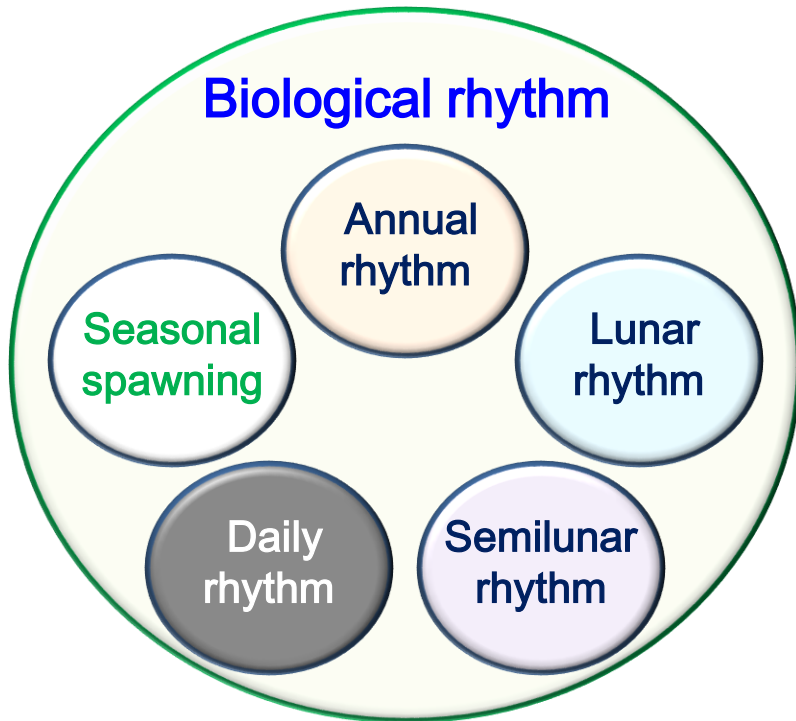
Bambooleaf wrasse

Winter breeder



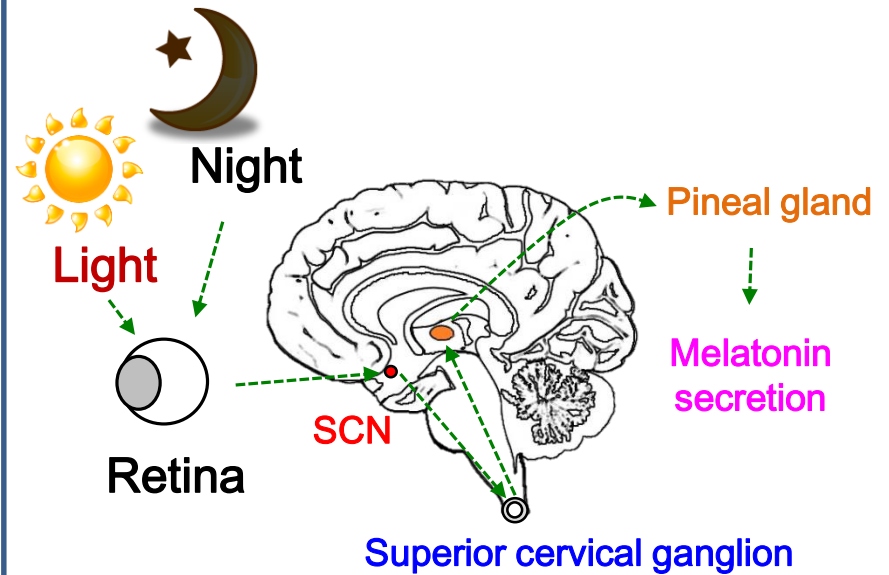
Ribbed Gunnel

❖ Biological rhythm



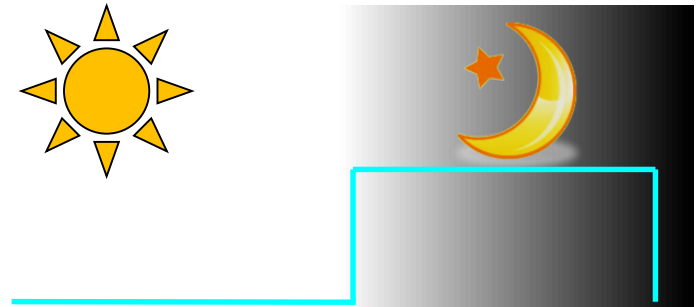
❖ Melatonin

: Main regulator of biological rhythm

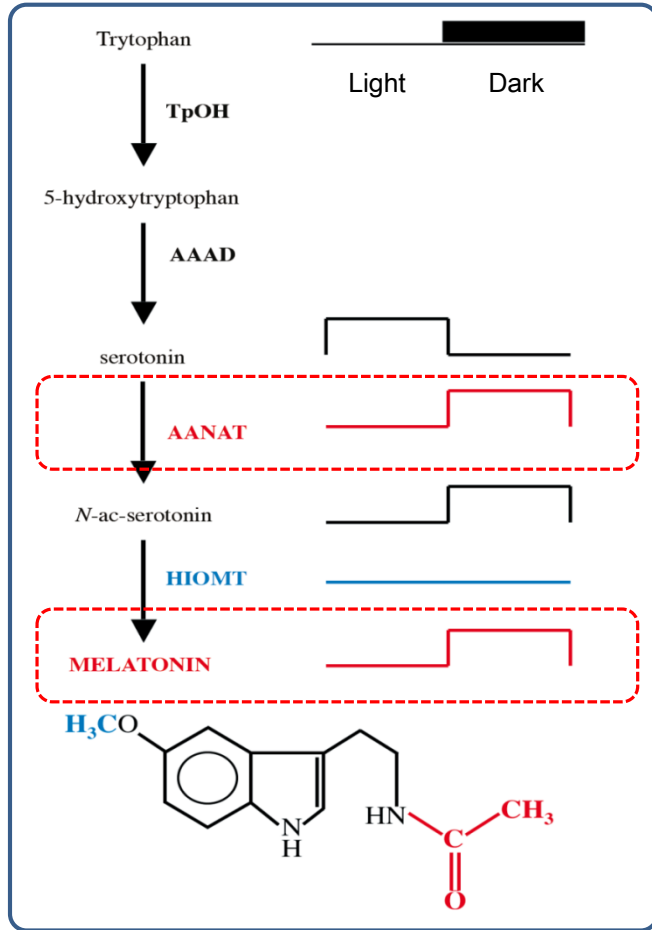


* SCN: Suprachiasmatic nucleus

: Melatonin is secreted during nighttime

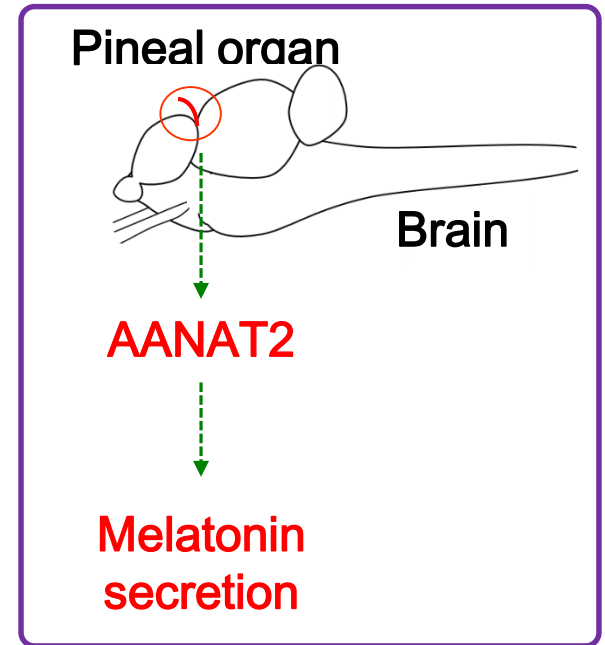
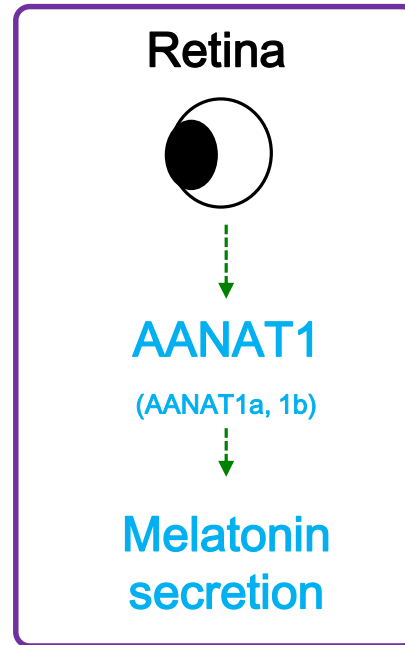


❖ Melatonin biosynthesis pathway



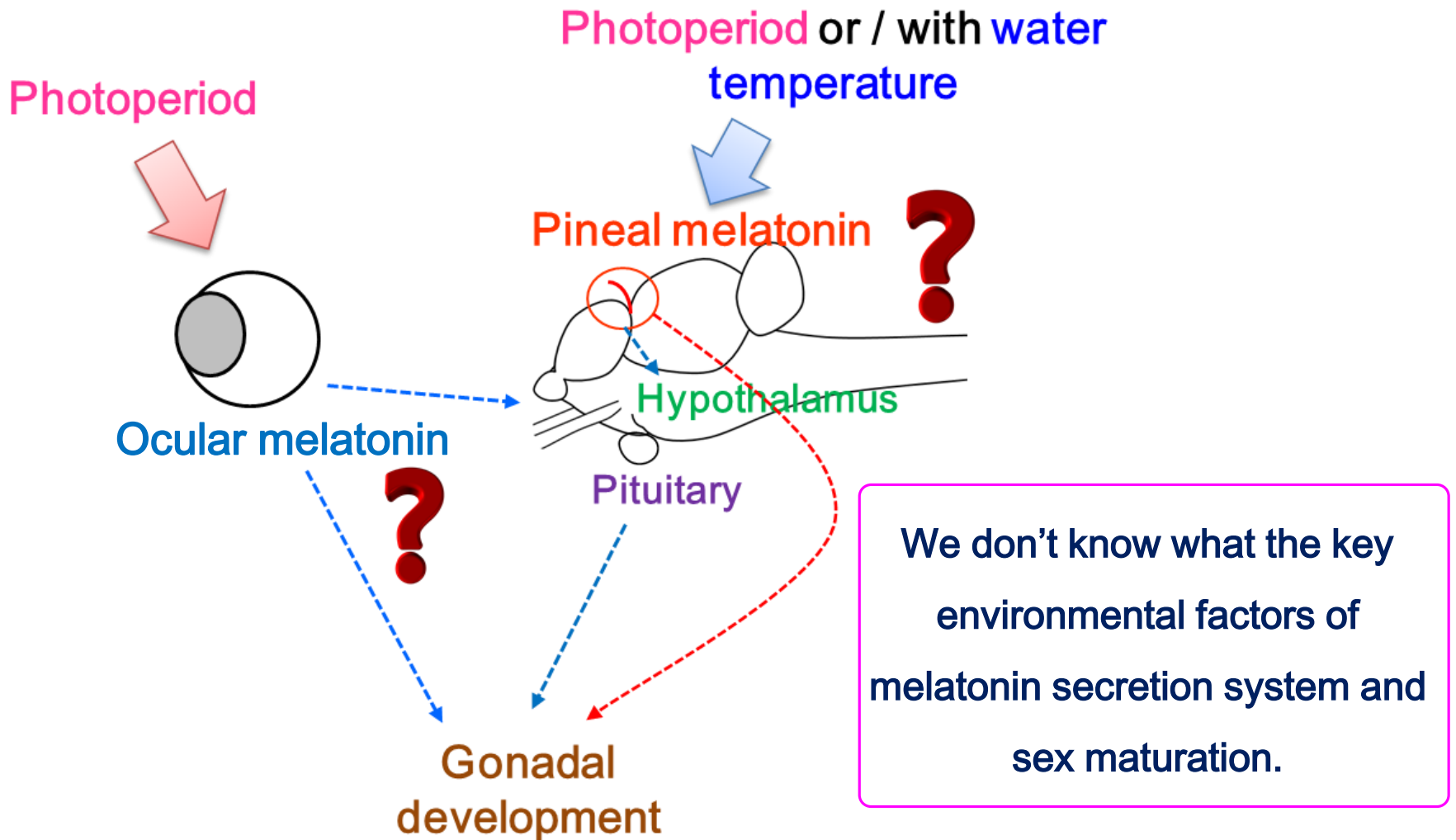
: **Aralkylamin *N*-acetyltransferase (AANAT)** is an enzyme that is involved in the day/night rhythmic production of melatonin

❖ The teleost fish...



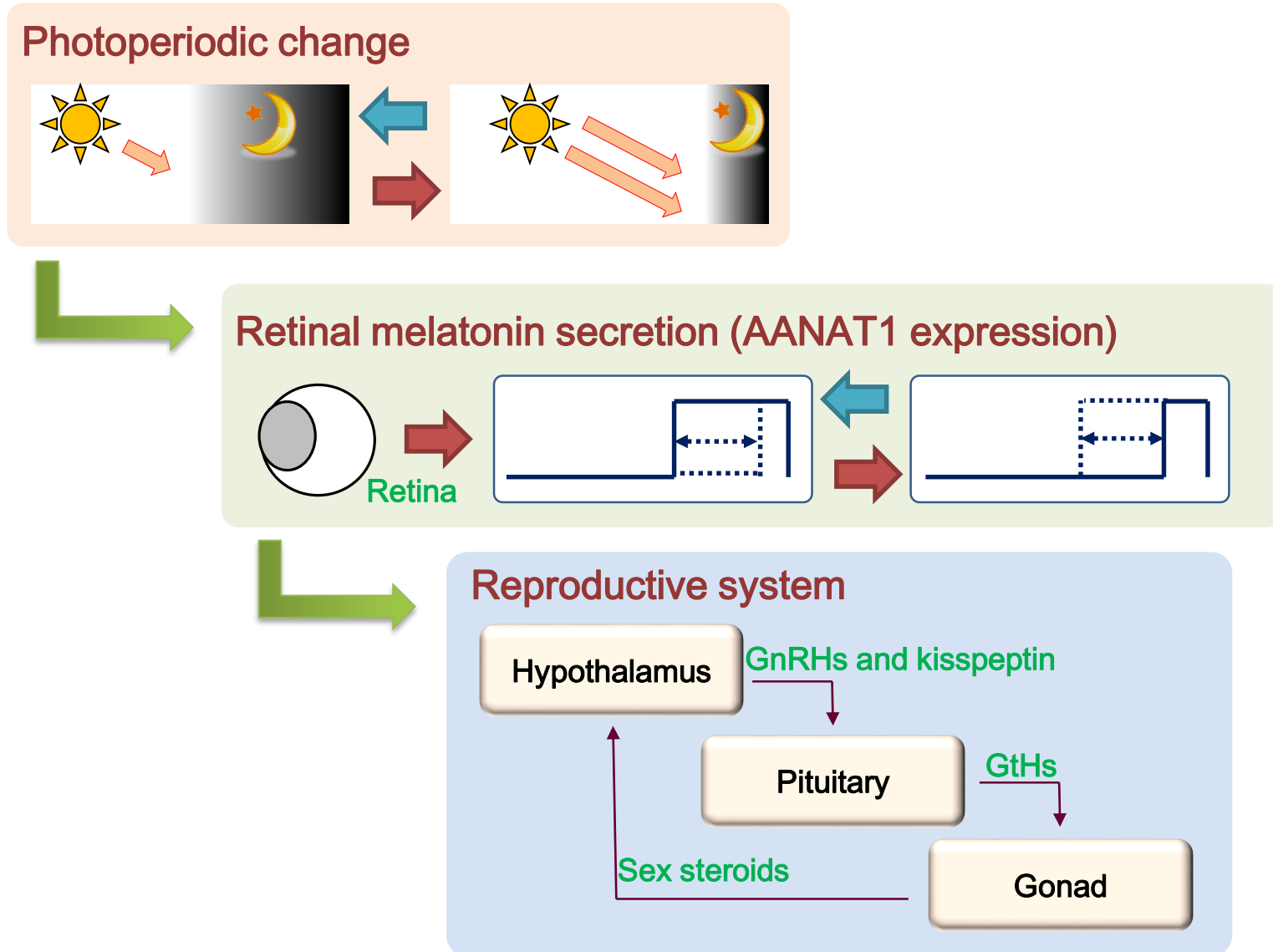
But...

: Melatonin secretion system is currently not clear in fish



In this study...

- We focused on photoperiodic change with retinal melatonin



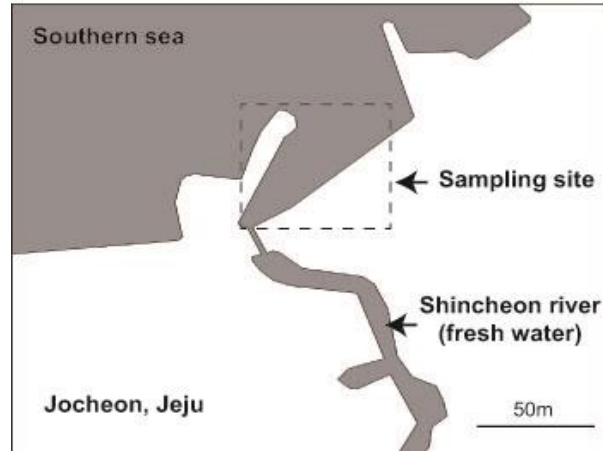
Materials & Methods

Grass puffer (*Takifugu niphobles*)



- Diurnal species
- Available genomic information

Sampling site



1. Annual reproductive rhythms

- ❖ Analysis: Gonadosomatic index (GSI) and Histological observation (H & E staining)

Real – time quantitative PCR (GnRH, Kisspeptin)

2. Tissue specific expressions of *aanat1* mRNA subtype

- ❖ Sampling tissues

- Nerves tissues:

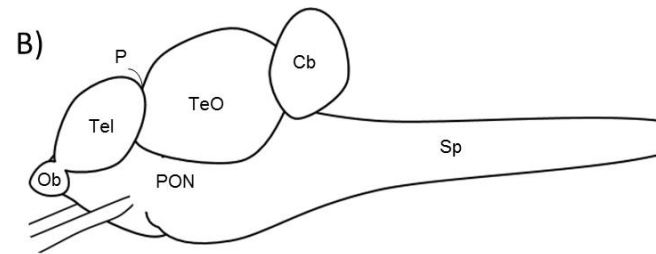
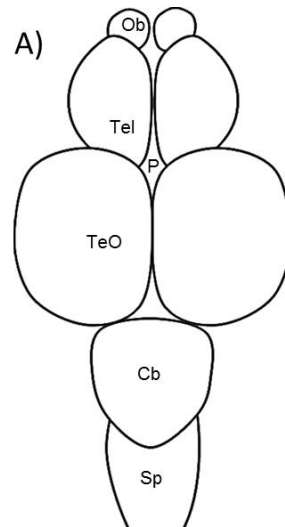
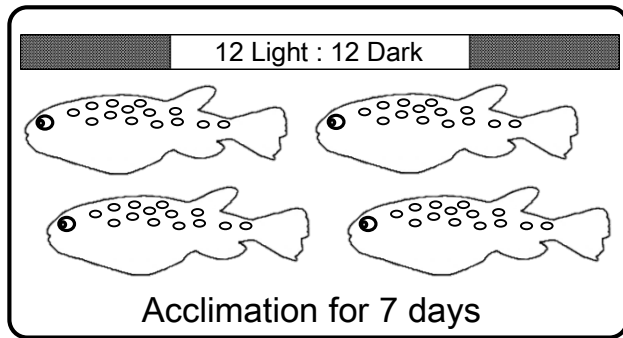


Fig. Diagram showing dorsal view (A) and sagittal plane (B) of the puffer fish brain. Ob, olfactory bulb; Tel, telencephalon; TeO, optic tectum; Cb, cerebellum; Mo, medulla oblongata; P, pineal gland; PON, preoptic nucleus; SV, saccus vasculosus.

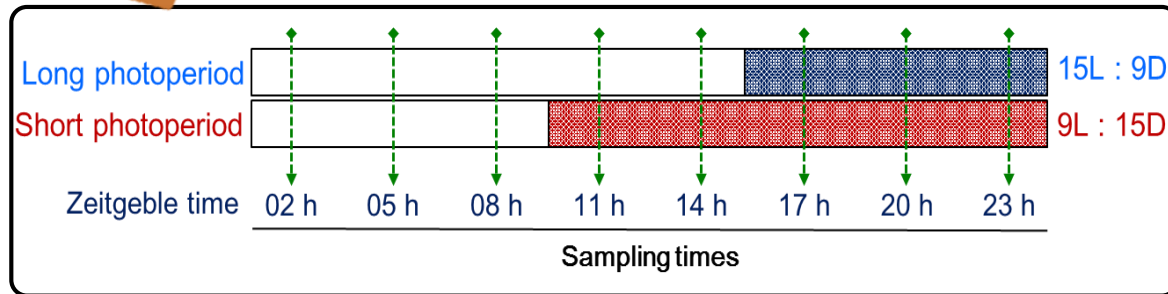
- Peripheral tissues: retina, gill, heart, liver, kidney, spleen, intestine, testis, ovary, muscle

- ❖ Analysis: RT-PCR and Real - time quantitative PCR

3. *aanat1* mRNA rhythms by artificial photoperiod

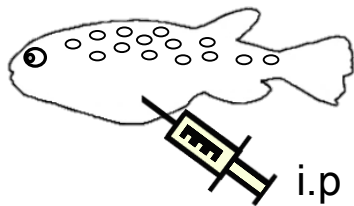


Sampling after acclimation of 7 days



- ❖ Sampling tissue: Retina
- ❖ Analysis: Real - time qPCR

4. Expression of reproductive related genes by melatonin treatment

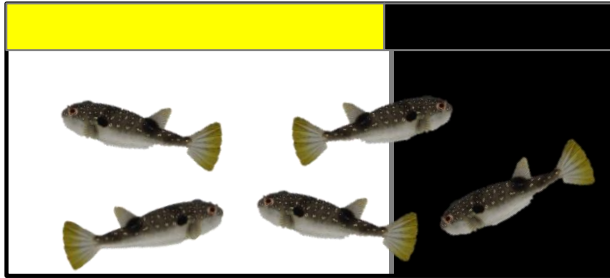


i.p (2 weeks)

- ❖ Reproductive related gene: Kisspeptin, GnRHs, GtHs
- ❖ Sampling tissue: Brain and pituitary
- ❖ Analysis: Real - time qPCR

5. Regulation of sex maturation by photoperiod manipulation

Long photoperiod (15L : 9D)



Short photoperiod (9L : 15D)

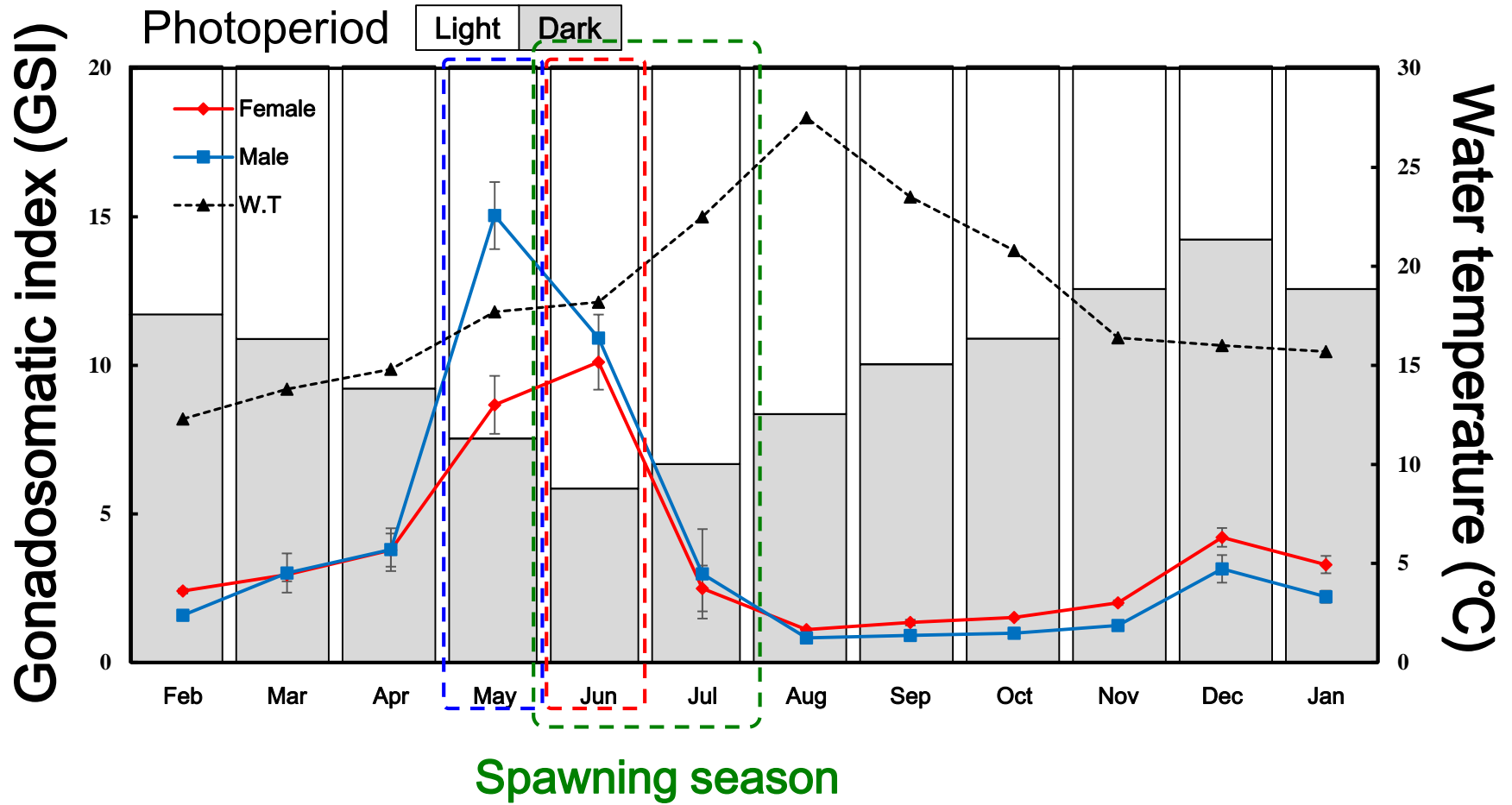


- ❖ Experimental period: 10 weeks
- ❖ Analysis: Gonadosomatic index (GSI) and Histological observation (H & E staining)

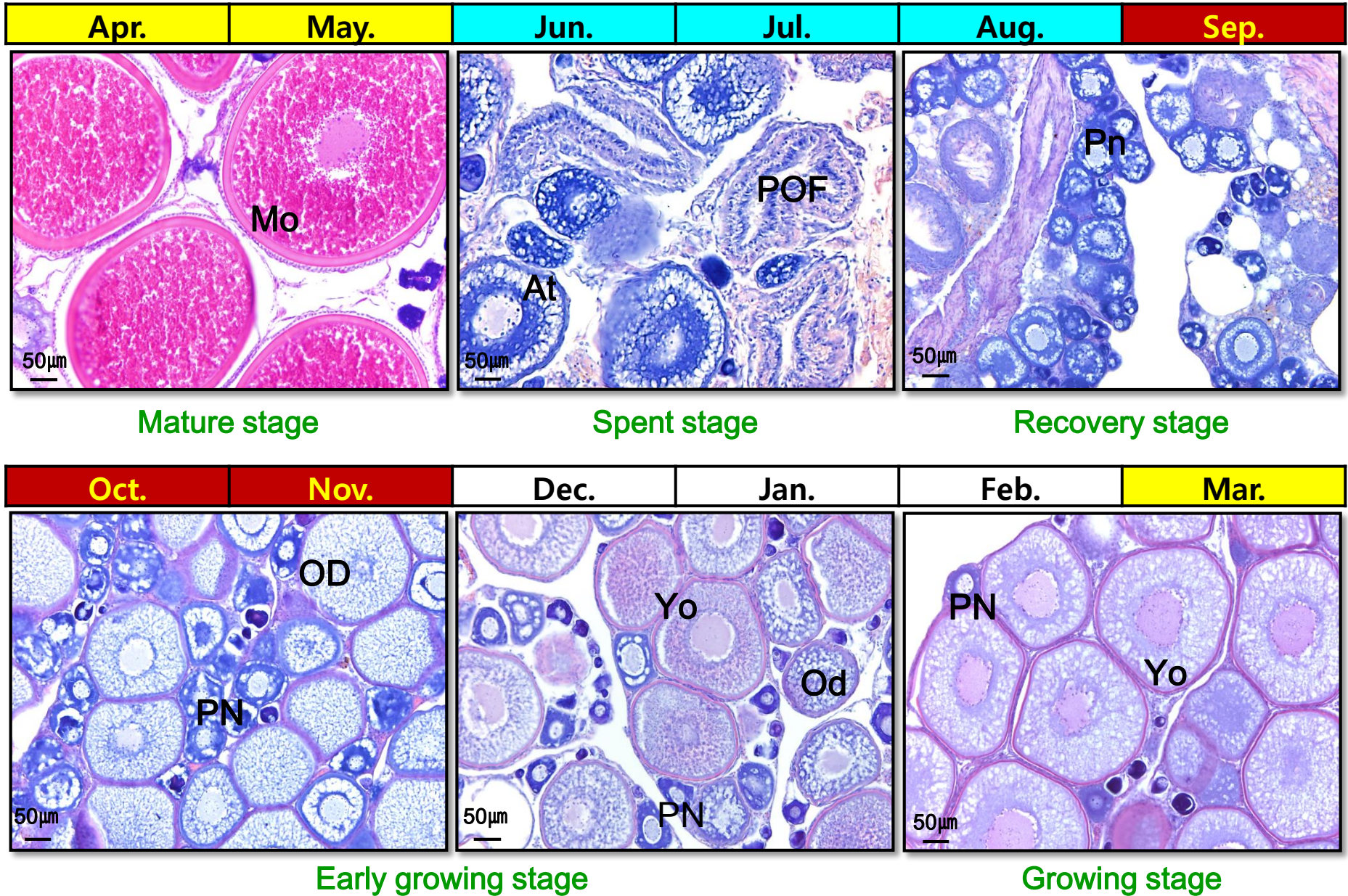


Results

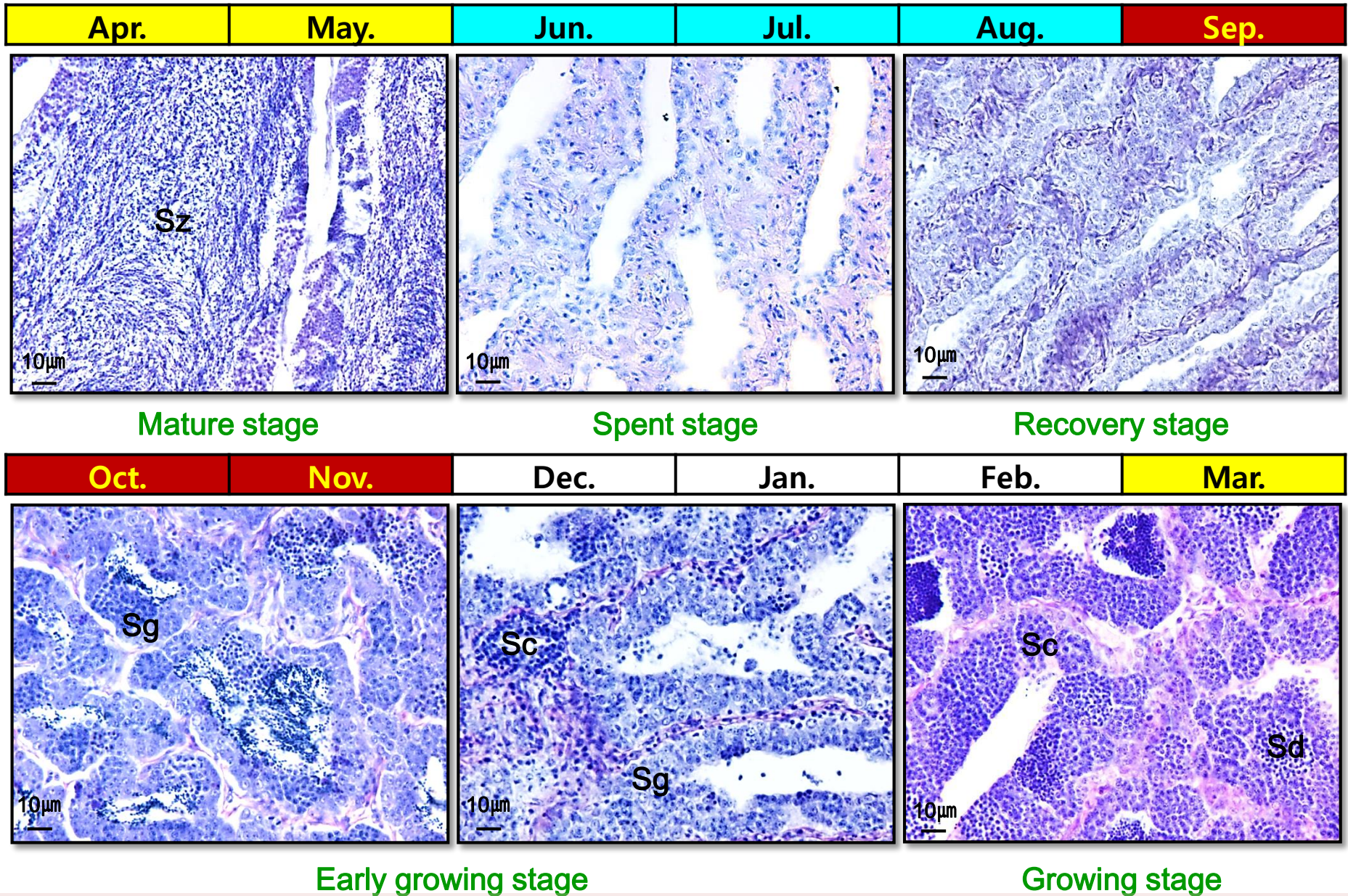
1. Gonadosomatic index (GSI)



2. Oogenesis of ovary in female grass puffer

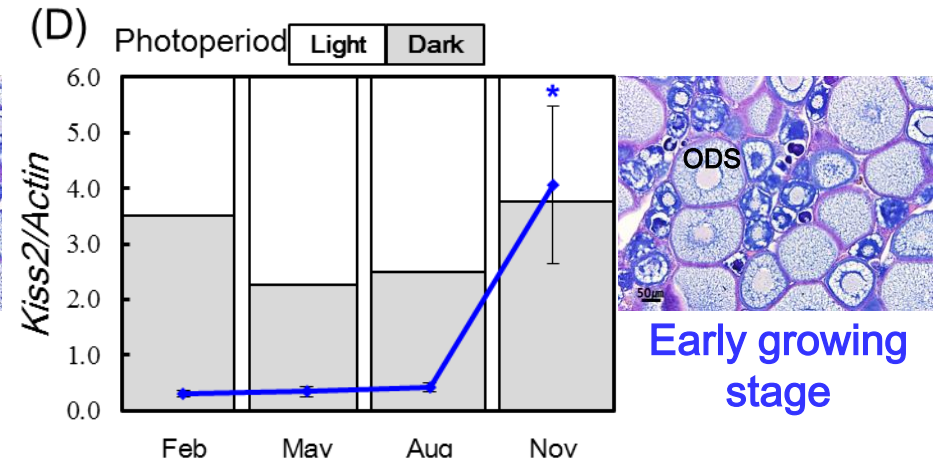
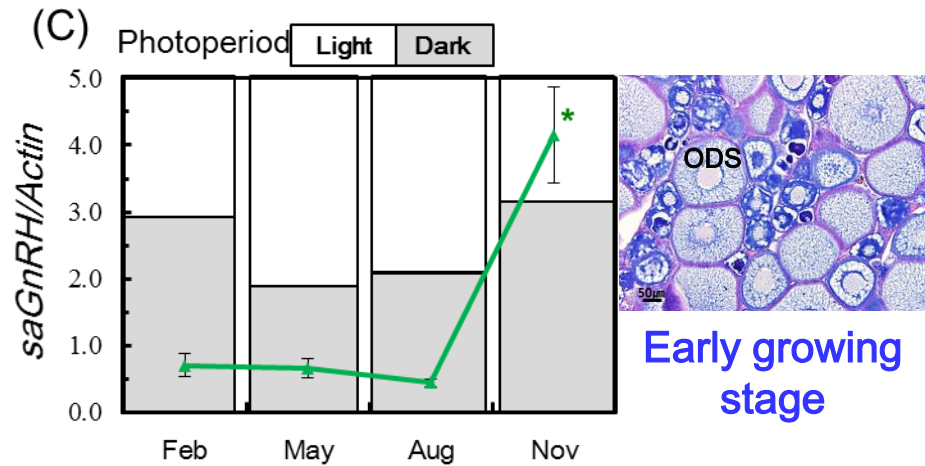
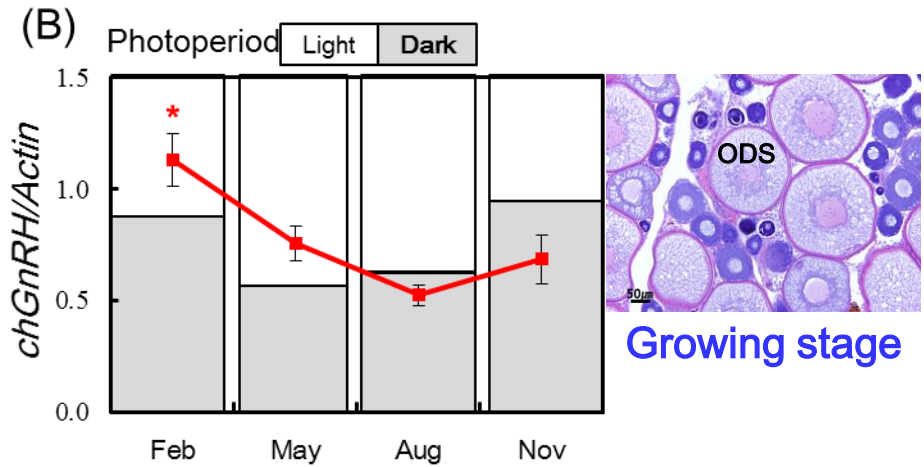
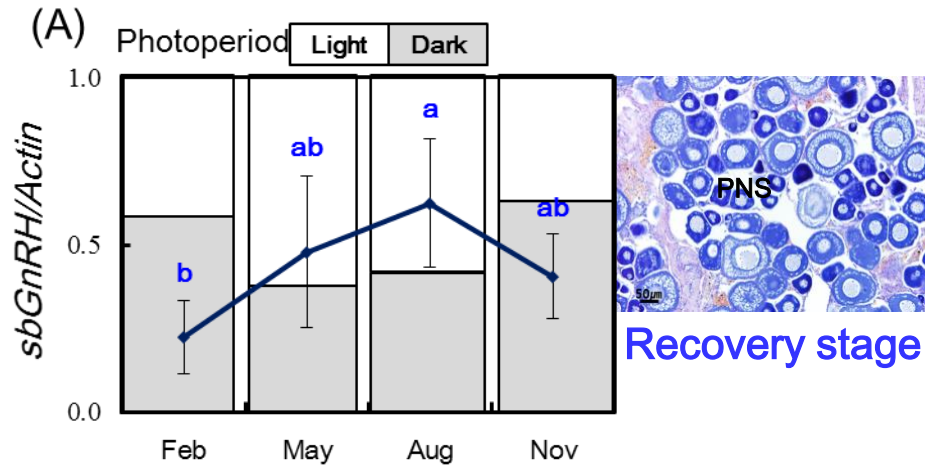


3. Spermatogenesis of testis in male grass puffer

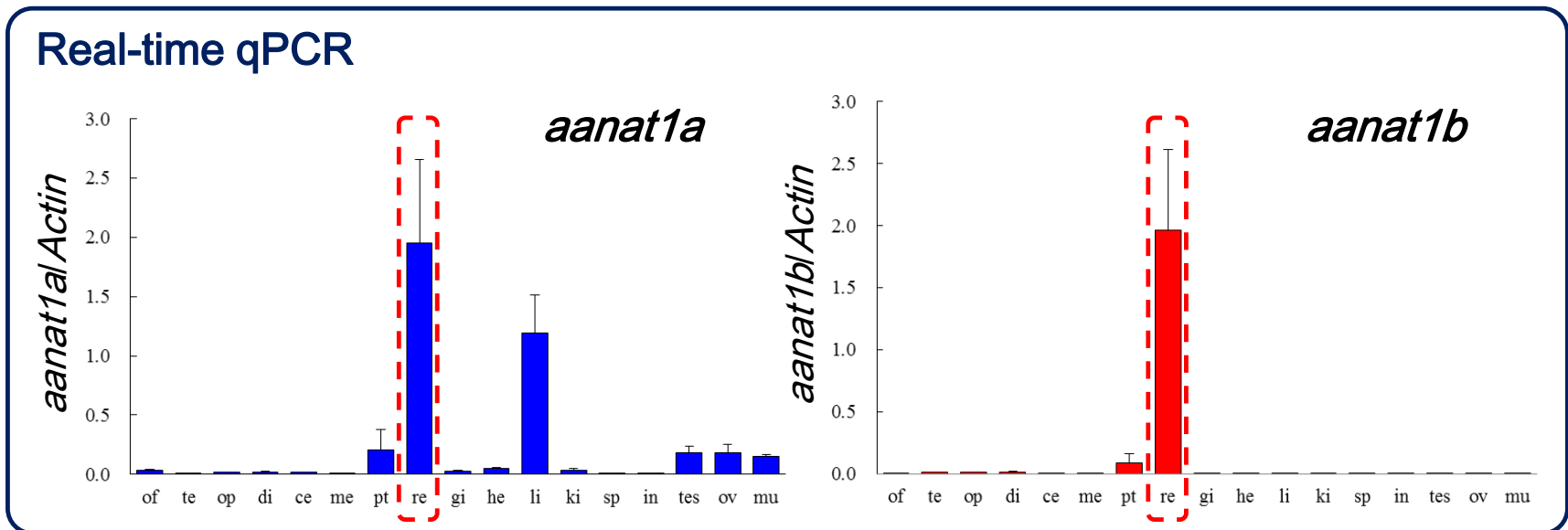
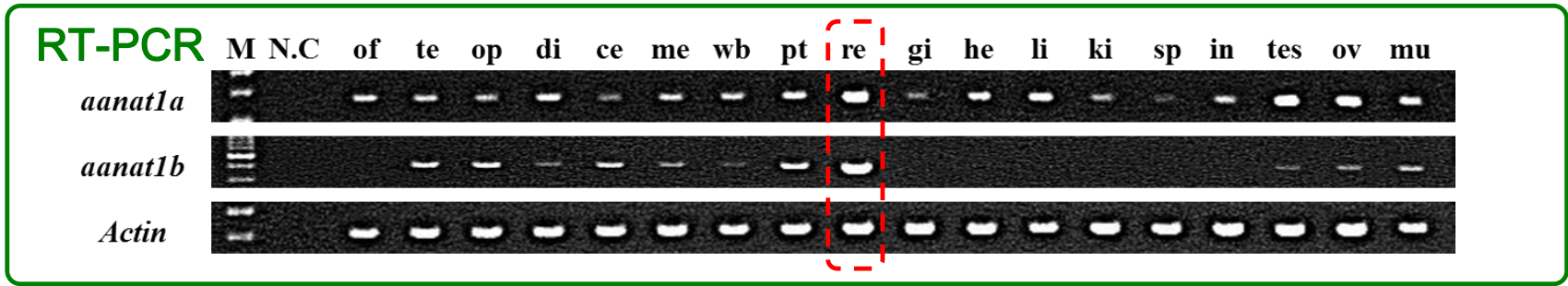


4. Seasonal expression of *GnRHs* and *Kiss2* mRNA

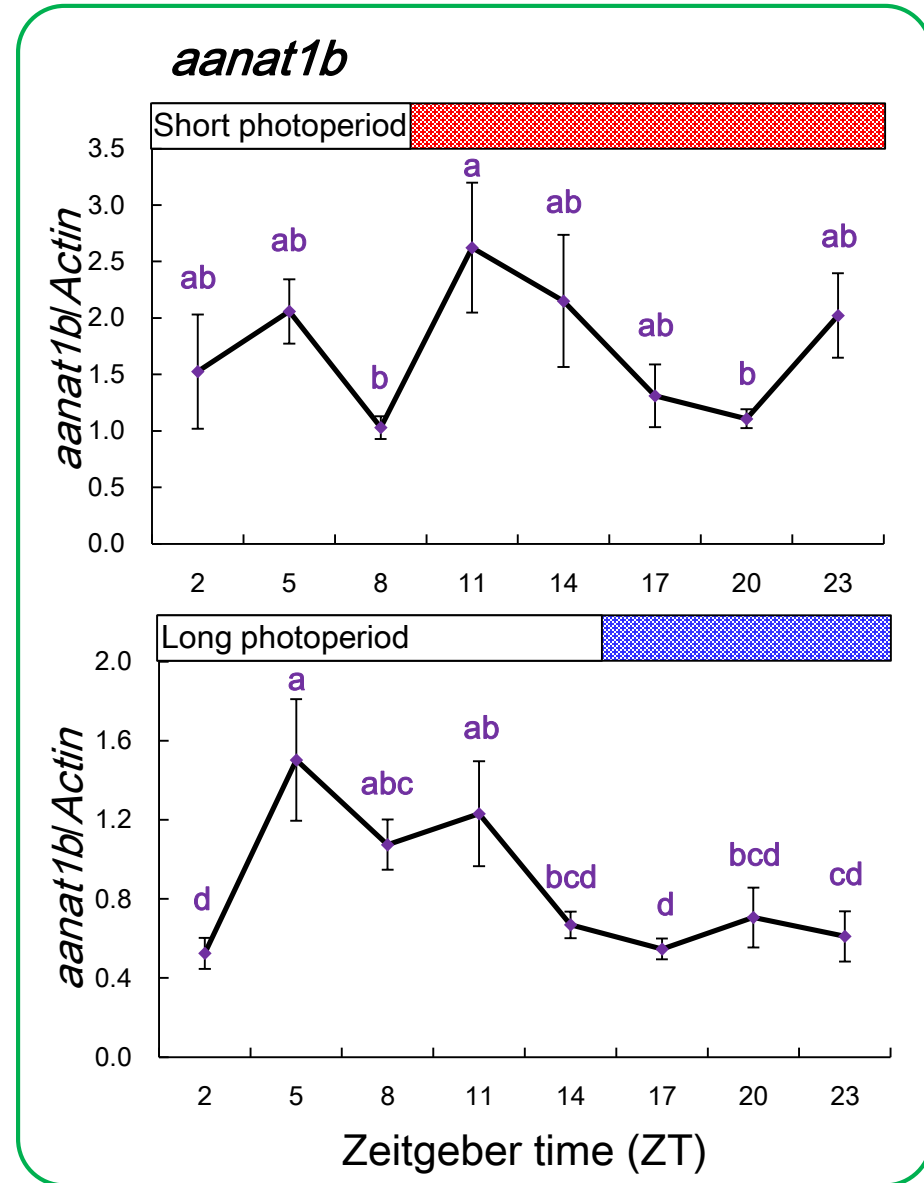
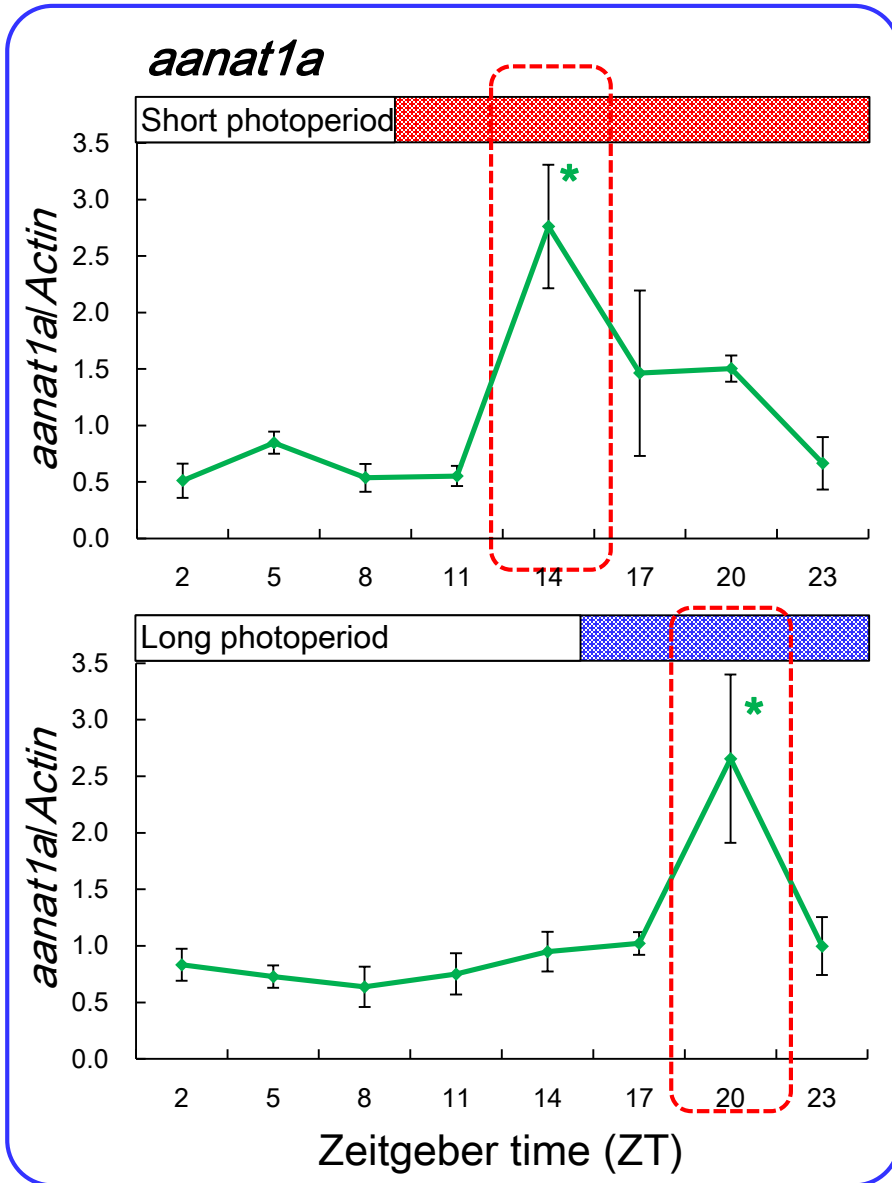
In Hypothalamus



5. Tissue specific expressions of *aanat1* mRNA subtype



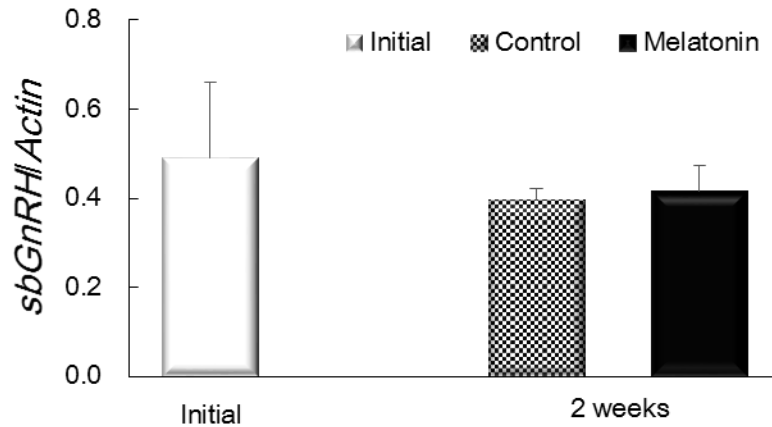
6. *aanat1* mRNA expression rhythms in retina under artificial photoperiod



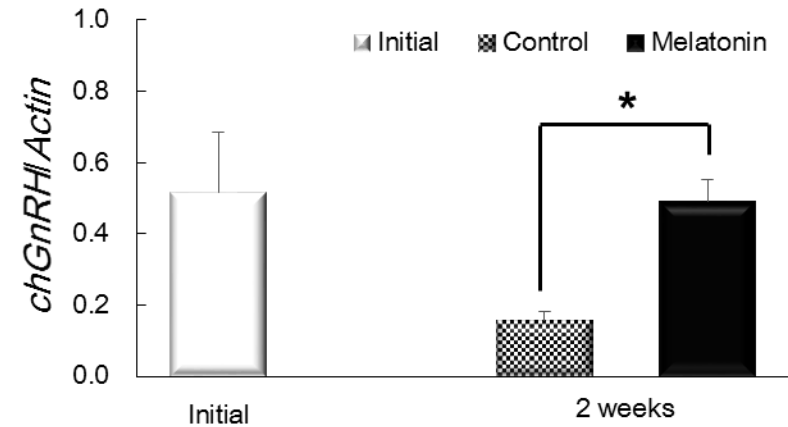
7. Reproductive related genes mRNA expression by melatonin treatment

In brain

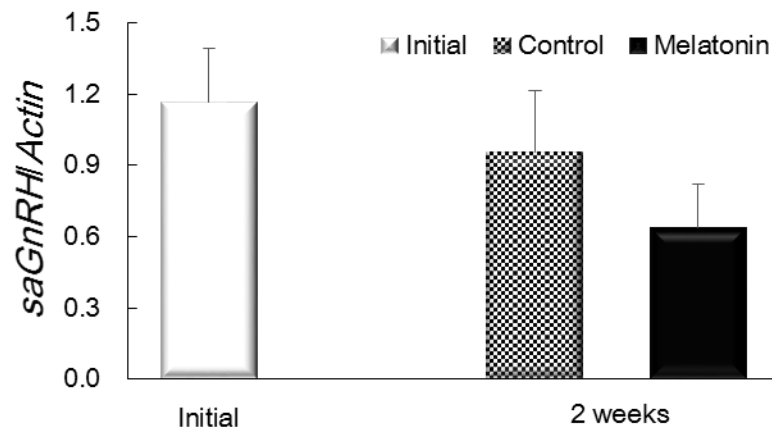
sbGnRH



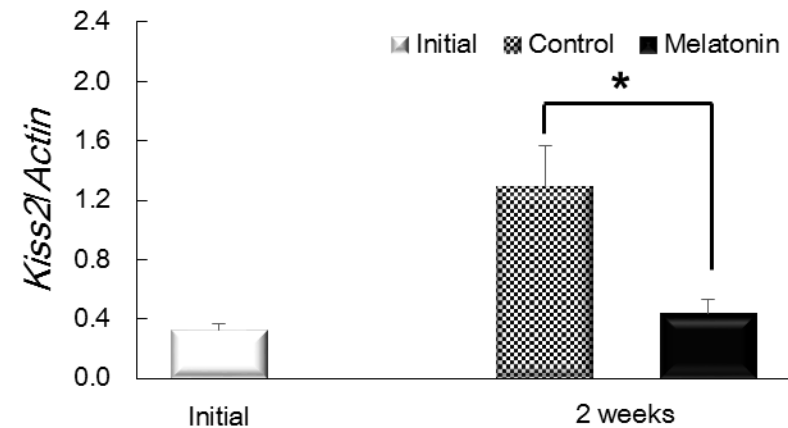
chGnRH



saGnRH



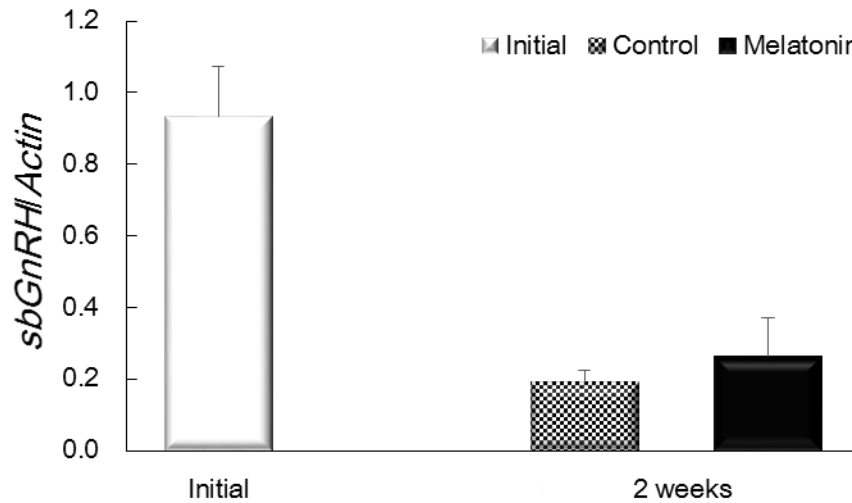
Kiss2



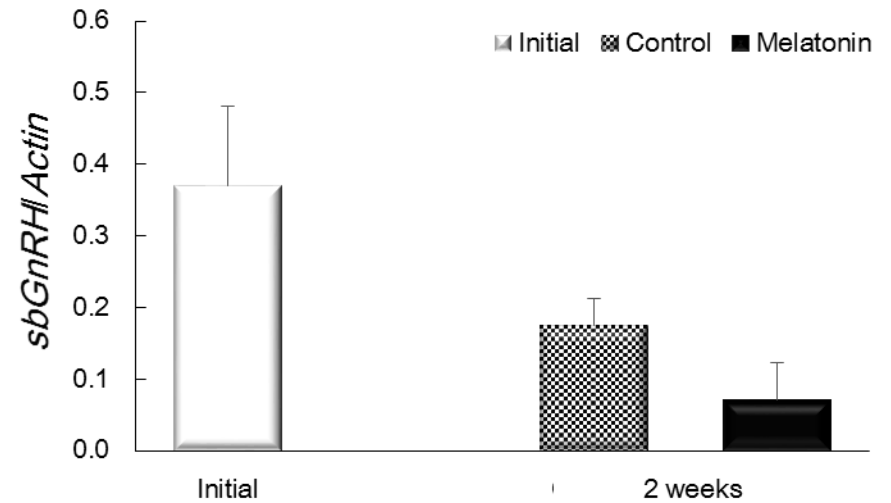
7. Reproductive related genes mRNA expression by melatonin treatment

In pituitary

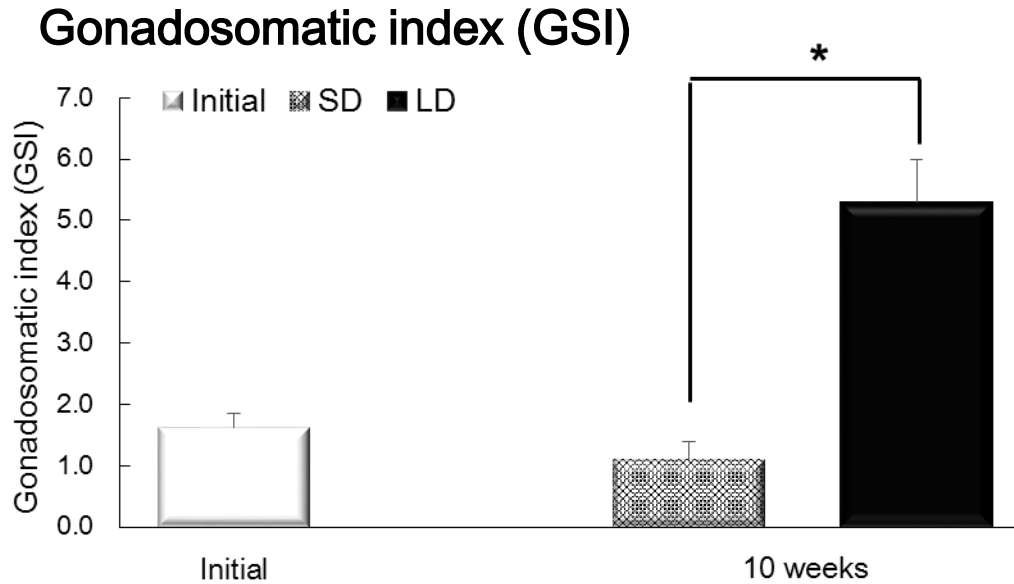
FSH



LH

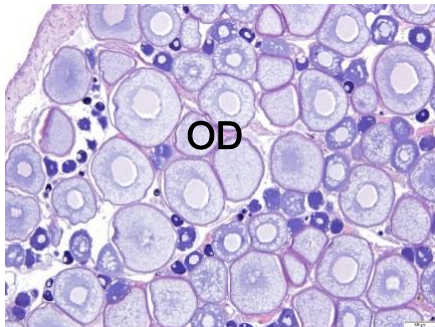


8. Regulation of sex maturation by photoperiod manipulation



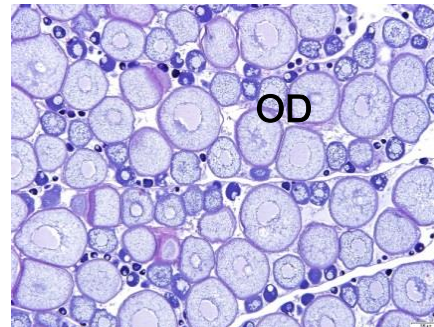
After 10 weeks

Initial



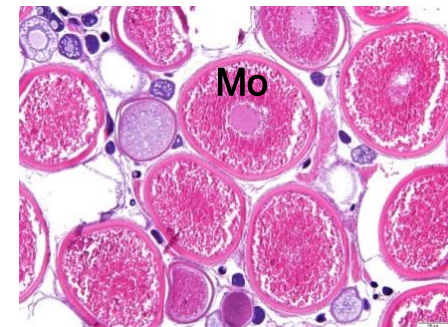
Early growing stage

Short photoperiod



Early growing stage

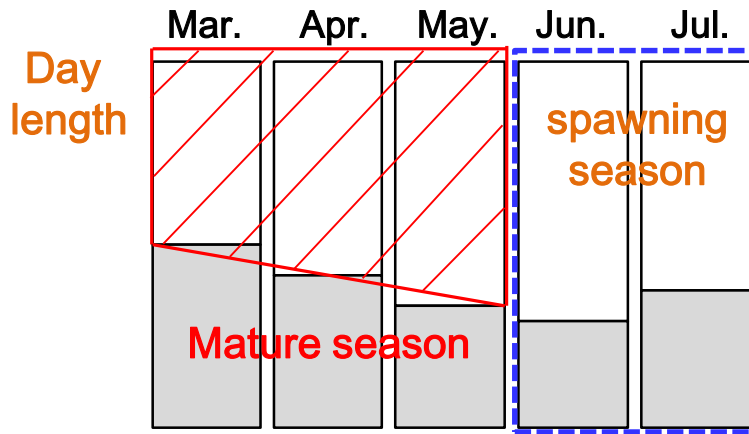
Long Photoperiod



Mature stage

Discussion

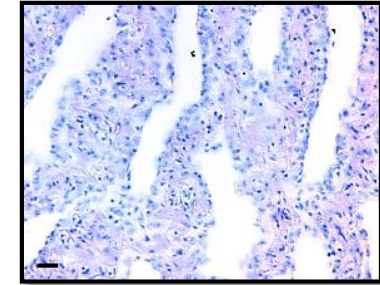
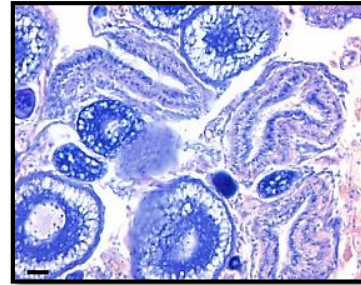
1. Spawning season of grass puffer



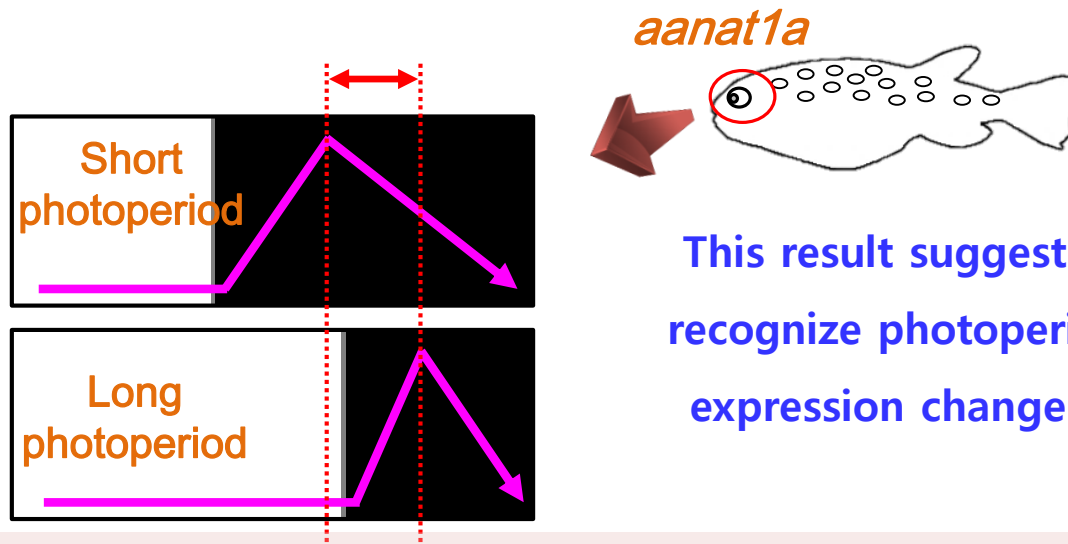
Histological observation

Ovary

Testis

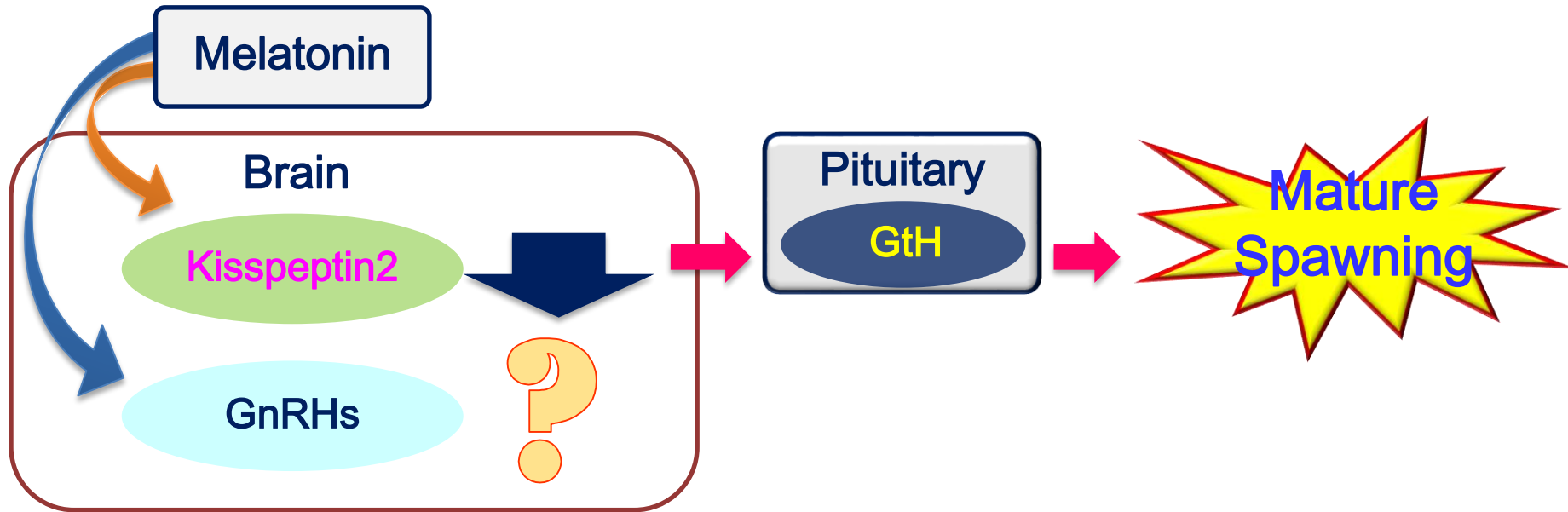


2. *aanat1a* mRNA expression artificial photoperiod conditions in retina

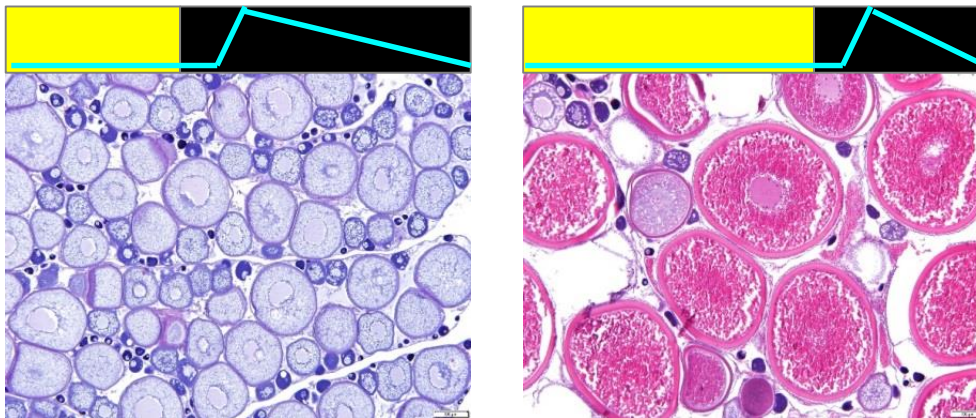


This result suggest that *aanat1a* mRNA was recognize photoperiod, and this was possible expression change by photoperiod change.

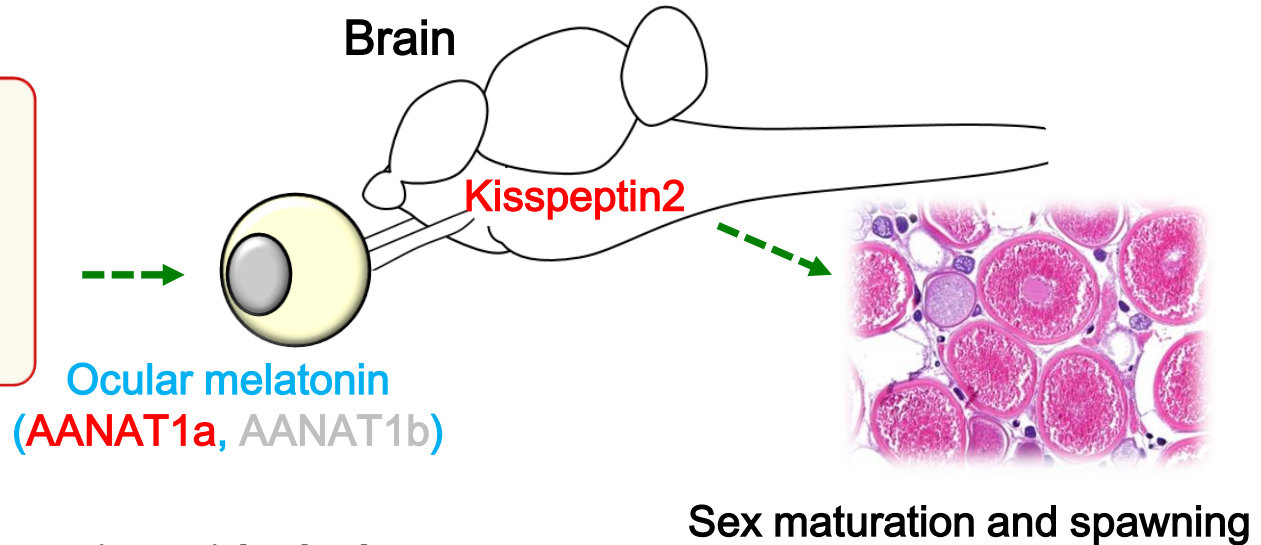
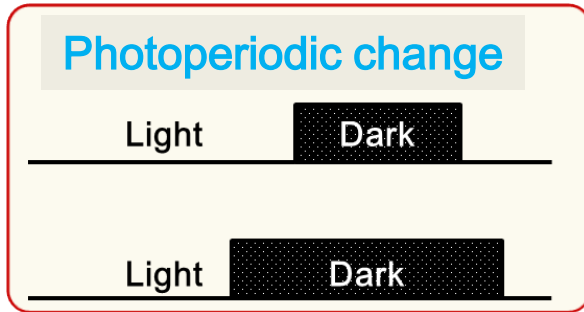
3. Effect of Melatonin on reproductive axis in grass puffer



4. Regulation of sex maturation by photoperiod control in grass puffer

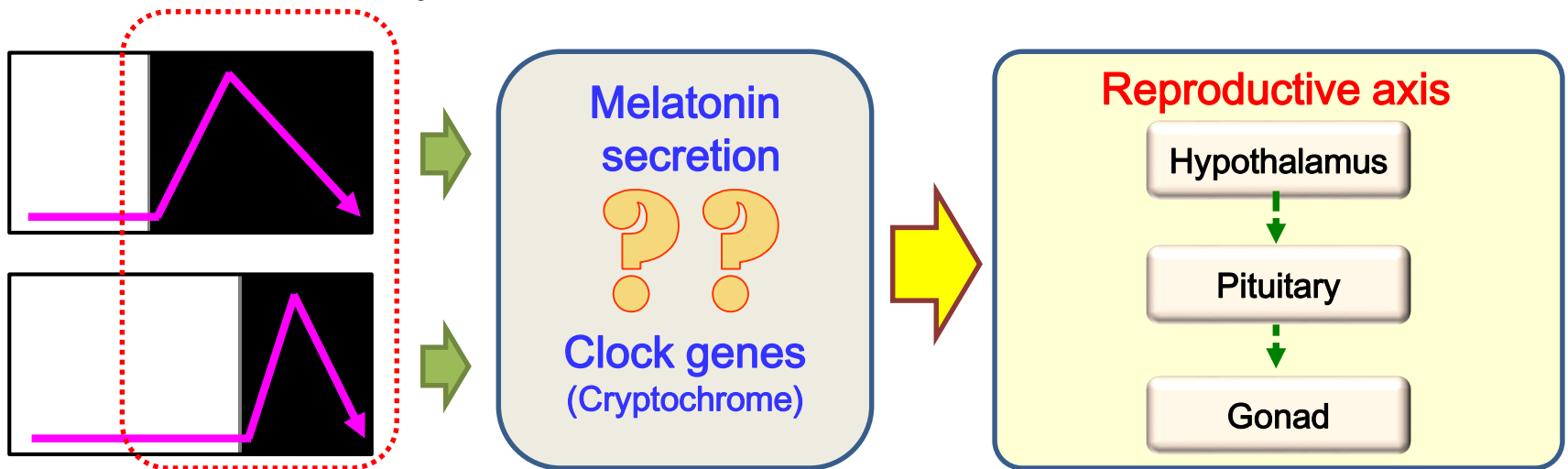


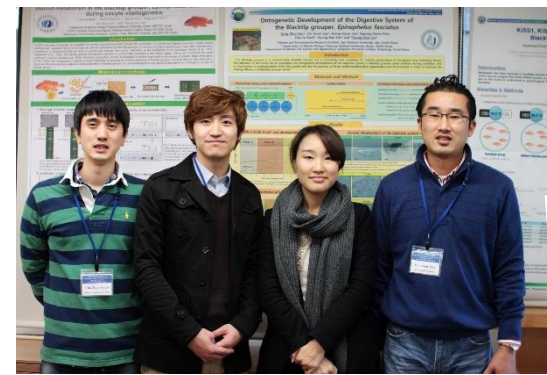
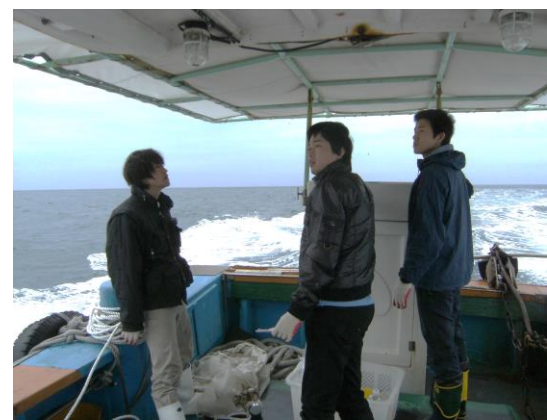
Summary



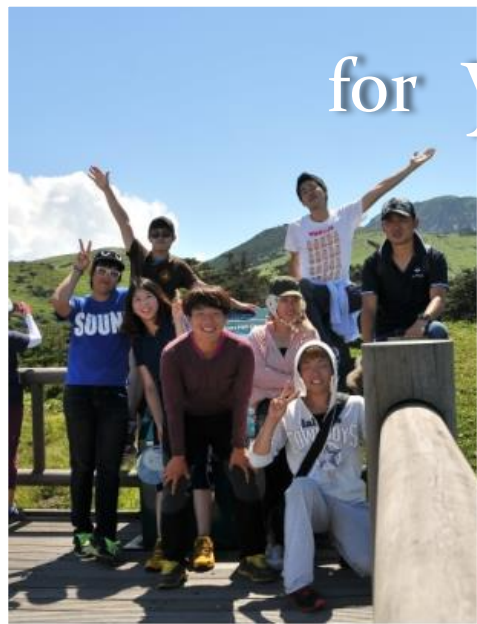
Further study...

: Relationship melatonin secretion with clock genes in reproductive system





Thank you



for your attention!

